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Potato Skin: A Potential Bio stimulating agent for used Motor Oil Bio degraders

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***Abstract*— *The potential of potato skin (PS) to enhance bioremediation of soil polluted with used motor oil was investigated gravimetrically for a period of 42 days. Polluted soil was amended with 5%, 10% and 15% (w/w) of PS. Loss of total petroleum hydrocarbon (TPH), microbial growth and germination indices were all investigated throughout the study period. At the end of 42 days, there was significant oil loss of 73.85% in the amended soil. Hydrocarbon-utilizing bacterial (HUB) counts were significantly higher($P \leq 0.05$) in the amended option ranging from 6.7 x 10^6 to 22.3 x 10^6CFU/g. The HUB isolated from the oil-contaminated soil were identified tentatively as belonging to the genera: Bacillus, Arthrobacter, Rhodococcus, Corynebacterium, Pseudomonas, Staphylococcus and Acinetobacter. Similarly, fungal counts ranged from 4.8 x 10^5 to 59.0 x 10^5 CFU/g. Aerobic fungi isolated were identified tentatively as Aspergillus niger, Aspergillus sp., Pennicillum sp., Phialophora sp., Cladosporium sp. and Verticillum sp. Germination index of 69.46% was recorded in the amended option. Oil loss and microbial growth were significantly higher ($P \leq 0.05$) in the amended option than the control option. Potato skin, therefore can offer a good alternative in bioremediation of soil polluted with used motor oil.*

Keywords*— *Bacteria, fungi, potato skin, bioremediation, used motor oil.

I. INTRODUCTION

Motor oil is a complex combination of hydrocarbons with other organic compounds, including certain organometallic compounds, it is used to lubricate the components of an automotive engine, to keep the engine and its whole operation working smoothly. Used motor oil (UMO) also contains impurities such as heavy metals, as well as polychlorinated bi-phenyls [1]. Used motor oil also contains toxins and mutagenic polycyclic aromatic hydrocarbons (PAHs), which accumulate gradually with miles due to direct fuel leakage into the motor oil, as well as the build-up of incomplete combustion products [2].

There have been growing use of motor oil due to the presence of different types of vehicles and machinery. Sadly, soil pollution is growing rapidly with used motor oil due to global growth in the use of petroleum products [3]. Spilling the used motor oils involves hydrocarbon damage to our natural environment with hydrocarbons [4]. Contaminants have been identified as being capable of accumulating and toxic to biological systems (plants and animals) [1].

A variety of revolutionary physical and chemical techniques are available for the remediation of hydrocarbon-contaminated areas, such as soil washing, vapor extraction, encapsulation and solidification/stabilization [5]. These approaches, however, are expensive and can be only partially effective. Furthermore, the field utilization of these intense techniques can be limited by public pressures [5].

It has been widely demonstrated that microorganisms possess inherent abilities to degrade hydrocarbons and UMO is not an exception. The degrading organisms utilize hydrocarbons as carbon source. It has been reported that while hydrocarbons are excellent carbon sources for organisms, they are incomplete foods in that they contain insufficient quantities of other nutrients such as nitrogen and phosphorus required for

microbial growth and activities [6]. Lack of essential nutrients, such as nitrogen and phosphorus, is one of the major factors affecting biodegradation of hydrocarbon by microorganisms in soil. Therefore, the addition of amendments (biostimulation), such as biochar, ash, pig manure, sewage sludge, is effective in lowering the metal and PAHs toxicity of soil and provides a slow release of nutrient sources such as N, P, K to enhance the bioremediation process [7]. The nitrogen amendment on microbial activity and/or petroleum hydrocarbon degradation has been widely demonstrated [8,9]. Organic wastes vary in their content of nitrogen and/or phosphorus and this reflects the extent they perform in their biostimulatory activities. This work was therefore carried out to assay for the biostimulatory potential of potato skin as an alternative biostimulation candidate for UMO-impacted soil based on its profile of nitrogen and phosphorus content.

II. MATERIALS AND METHODS

Collection of Samples

The soil sample was collected from the Faculty of Agriculture farm, University of Nigeria, Nsukka, Southeast Nigeria in sterile polythene bags at a depth of 0-15 cm from different sampling sites and transported to the laboratory for analysis. Used motor oil was collected from the Mechanic village, Nsukka, Enugu state, Nigeria. Potatoes were bought from Ogige Market, Nsukka and peeled to take their skin. Seeds of cucumber (*Cucumis sativus*) were obtained from the Department of Crop Science, University of Nigeria, Nsukka.

Determination of Physicochemical Properties of Soil and Potato Skin

Physicochemical properties of soil and potato skin such as particle size distribution, percentage moisture content, pH, total organic carbon (%TOC), % nitrogen content and total phosphorus were analyzed following standard protocol [10]

Soil Preparation for Bioremediation

A 1kg quantity of soil (sieved with 2 mm mesh size) was placed in sterile polythene bags and polluted with 10% (v/w) UMO, and left undisturbed for 48 hours. The polluted soil was amended with air-dried and pulverized potato skin at the concentrations of 5%, 10% and 15% w/w. Soil neither polluted nor amended served as the positive control while polluted soil without amendment served as the negative control. The moisture content of the soil was adjusted to 60% water holding capacity by the addition of sterile distilled water (50 ml, three times weekly) and the set-up incubated at room temperature (28 ± 2°C). Periodic triplicate sampling from each set-up was carried out at 7-day intervals for isolation and enumeration of microorganisms (fungi and bacteria) and determination of residual UMO.

Determination of Extraction Efficiency of Different Solvents for Diesel Oil

The extraction efficiency of three organic solvents, namely: dichloromethane, diethylether and n-hexane for used motor oil was predetermined in order to examine the rate at which the solvents would be able to extract the UMO pollutant from polluted soil. Extraction efficiency study was carried out gravimetrically. Briefly, a 20 g portion of soil mixed with 2 mL used motor oil was homogenized and left for two hours in a 250 mL flask. Thereafter, the soil-oil mixture was mixed with 80 mL of the different solvents and the set-up shaken for eight hours at 180 rpm. The solution was then filtered using a Whatman No 4 filter paper and the weight of the extracted oil recorded. The extraction efficiency of the organic solvents for UMO was then determined by weight difference following the formula [11]. The experiment was carried out in triplicates.

$$\text{Extraction efficiency} = \frac{\text{Weight of 2 mL UMO} - \text{Weight of oil extracted from soil}}{\text{Weight of 2 mL diesel oil}} \times 100$$

Soil Preparation for Bioremediation Study

A 1 kg quantity of the sieved soil was placed in sterile polythene bags and 10 % (v/w) of UMO was added, mixed thoroughly, and left undisturbed for 48 hours. After two days, 5%, 10% and 15% (w/w) pulverized potato skin were respectively, introduced into the UMO-polluted soils and mixed thoroughly. Soil sample contaminated with 10% (v/w) UMO without amendment served as control. The moisture content of the soil was adjusted to 60% water holding capacity by the addition 50 mL of sterile distilled water (three times weekly) and the set-up kept at room temperature (28±2°C). The experiment was set up in triplicates.

Determination of Percentage Bioremediation

Periodic sampling from each polythene bag was carried out every seven days in order to determine residual UMO. Gravimetric method [12] was modified slightly and employed in the determination of UMO present in both the unamended control soil and all the amended microcosms. Composite polluted soil samples weighing 5 g were put in a 50 mL flask and 10 mL of diethyl ether was added. Diethylether was used

because it gave the highest extraction efficiency (see result section). The set-ups were shaken with a rotary shaker at 180 rpm for two hours to allow for an efficient and complete oil extraction with diethyl ether. The mixture was then filtered with a whatman No 4 filter paper. The filtration was done repeatedly two times to ensure complete extraction of the liquid phase. The filtrate was diluted by adding 50 mL of diethylether to 1 mL of the extracted UMO and the absorbance of the solution measured at 460 nm (Shimadzu UV 1800) using diethylether as blank. The total petroleum hydrocarbon (TPH) was estimated by extrapolating from a standard curve derived from different concentrations of fresh UMO diluted with diethylether. Percent remediation (R) was calculated using the following formula:

$$R = \frac{TPHi - TPHr}{TPH} \times 100$$

Where TPHr and TPHi are residual and initial TPH concentrations

Enumeration and Identification of Microorganisms

The indigenous hydrocarbon-degrading flora was enumerated following standard bacteriological and mycological methods. For bacteria, 10-fold serial dilutions were made from oil-polluted soils undergoing treatment and 0.1mL aliquot of the appropriate dilutions were spread on nutrient agar plates. Triplicate plates were incubated at 30 °C for 24 h before the bacterial colonies were counted. Hydrocarbon utilizing bacteria (HUB) in the soil samples were enumerated using modified mineral salts medium [13]: 1.8 g K_2HPO_4, 0.1 g $CaCl_2$, 0.2 g $MgSO_4.7H_2O$, 1.2 g KH_2PO_4, 0.01 g $FeSO_4.7H_2O$, 0.1 g NaCl, 20 g agar, in 1000 ml distilled water, pH 7.4, using the vapour phase transfer method [14]: briefly, a filter paper saturated with sterile UMO was aseptically placed on the inside of the inverted petri dishes of the inoculated mineral salt agar and the culture plates were incubated at 28 °C for 7 days [15]. Morphologically distinct hydrocarbon-utilizing bacteria (HUB) were randomly picked and pure isolates were obtained by repeated sub-culturing on nutrient agar. The bacterial isolates were identified tentatively by Gram reaction and biochemical characteristics.

For fungal enumeration and identification, ten-fold serial dilutions were made by suspending 10 g of treated soil in 90 ml of sterile distilled water. The soil suspension was shaken vigorously and allowed to settle. A 0.1 mL aliquot of the appropriate suspensions (10^{-3} to 10^{-6}) were spread on SDA plates and incubated at 25 °C for 4 days. Counts were taken from the plates as colony forming units/g. The fungal isolates were characterized by slide culture and microscopic techniques and identified by the schemes of Tsuneo [16].

Germination Toxicity Test for Remediated soil

Toxicity of the remediated soil was assessed using germination test of Jaqueline *et al.*, [17]. *Cucumis sativus* (cucumber) was used in this study owing to its sensitivity to hydrocarbon in soil. Briefly, thoroughly mixed treated soil samples were placed in 100 × 15 mm Petri dishes. Ten viable seeds of *Cucumis sativus* were placed evenly throughout each Petri dish and covered with dry sand. Three replicates of the samples were prepared and 10 mL distilled water was sprinkled daily. Soil neither polluted nor amended served as the positive control while polluted soil without amendment served as the negative control. At the end of 21 days, the number of seedlings that emerged from the surface of the sand were counted and recorded. Their root lengths were measured to the nearest mm using a meter rule. Germination index of cucumber seed on the remediated soil was calculated using the formula of Millioli *et al*. [18]

$$GERMINATION\ INDEX(\%) = (\%\ seed\ germination \times \%\ root\ elongation) \div 100$$

$$\%\ Seed\ Germination = \frac{Number\ of\ seed\ germination\ on\ polluted\ soil}{Number\ of\ seed\ germination\ on\ positive\ control\ soil} \times \frac{100}{1}$$

$$\%\ Root\ Elongation = (GERm \div GERCm) \times 100$$

Where, GERm = root length of seedling that germinated on treated soil, GERCm = root length of seedling that germinated on control soil.

Statistical Analysis

The data obtained in the present study were subjected to analysis of variance (ANOVA). Relationship between variables and comparison of means of the different treatments were tested for level of significances at $P \leq 0.05$ using least square difference and post-hoc multiple comparison tests. The data analysis was performed using SPSS.

III. RESULTS

Physicochemical Properties of Soil and Potato Skin

The physicochemical properties of the soil and potato skin used in this study are presented in **Table 1.** The soil textural class was clayey loam and it had nitrogen (0.15%), organic carbon (2.49%), phosphorus (10.64%), moisture (15.38%), pH (7.03%), sand (31.5%), silt (19.75%) and clay (48.75%). The soil used for bioremediation had C: N ratio of 16:6. The potato skin had nitrogen content of 0.602%, phosphorus content 24.08%, organic carbon content 29.93%, moisture content of 48.68% and pH values of 6.8.

Table 1. Physicochemical properties of soil and organic wastes used for bioremediation.

	Nitrogen (%)	Phosphors (%)	Moisture content (%)	Organic carbon (%)	pH	Sand (%)	Silt (%)	Clay (%)
soil	0.15±0.02	10.64±1.50	15.38±0.30	2.49±1.10	7.03±1.15	31.5±0.6	19.75 ± 1.95	48.75 ± 2.75
Potato skin	0.602±0.1	24.08±2.0	48.60±3.5	29.93±0.91	6.8±0.49	-	-	-

Extraction Efficiency of Solvents for Crude Oil

The level of extraction of crude oil by three solvents namely: n-hexane, dichloromethane and diethylether eight hours post-pollution were 85.5%±0.07, 86.7% ±0.76 and 89.0% ±1.97

Determination of TPH Loss (Bioremediation)

The level of crude oil loss in both the control soil and polluted soil amended with 5% PS over a 42-day period is presented in **Figure 1**. Percentage oil loss in the amended soil ranged from 27.18% to 60.77%. Oil loss in the control option ranged from 15.67% to 17.56%.

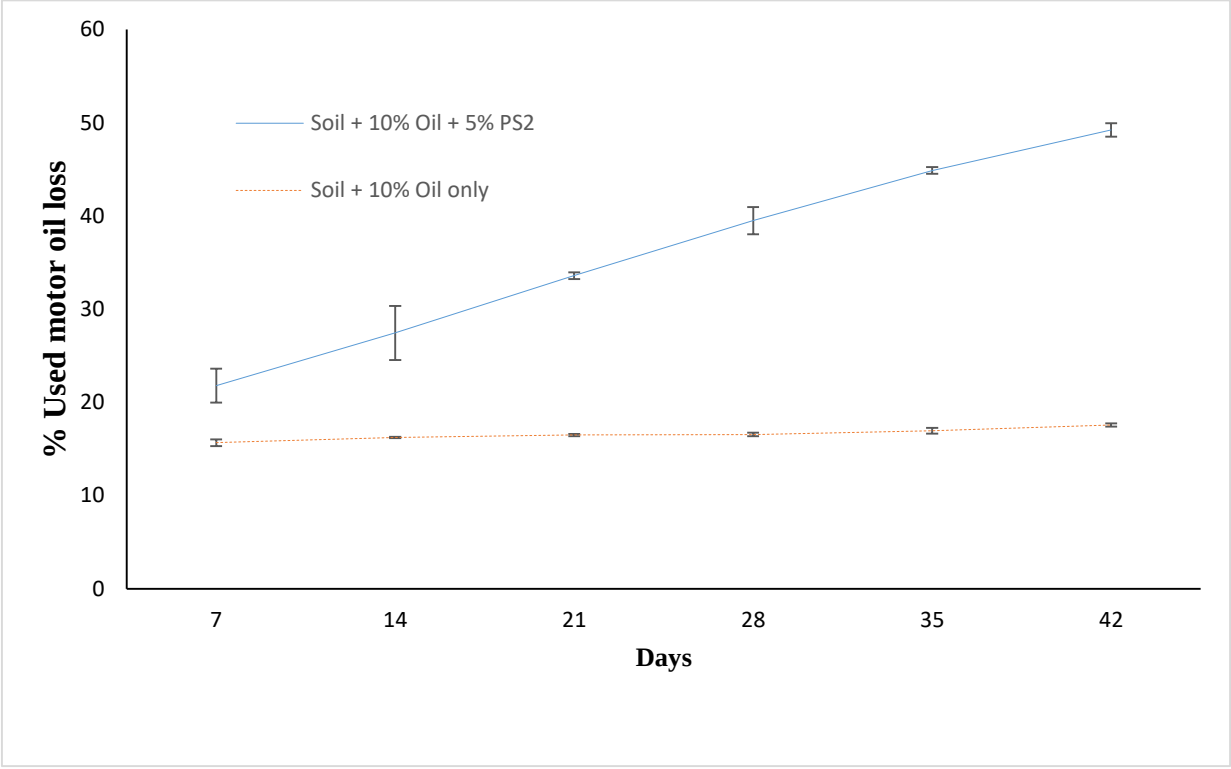

Fig.1: Bioremediation in soil polluted with 10% used motor oil and amended with 5% (w/w) PS. Error bars indicate standard errors (n = 3).

Figure 2 shows the level of oil loss in both the control soil and polluted soil amended with 10% PS. Oil loss in the PS-amended soil ranged from 31.54% to 63.84% within the 42-day period.

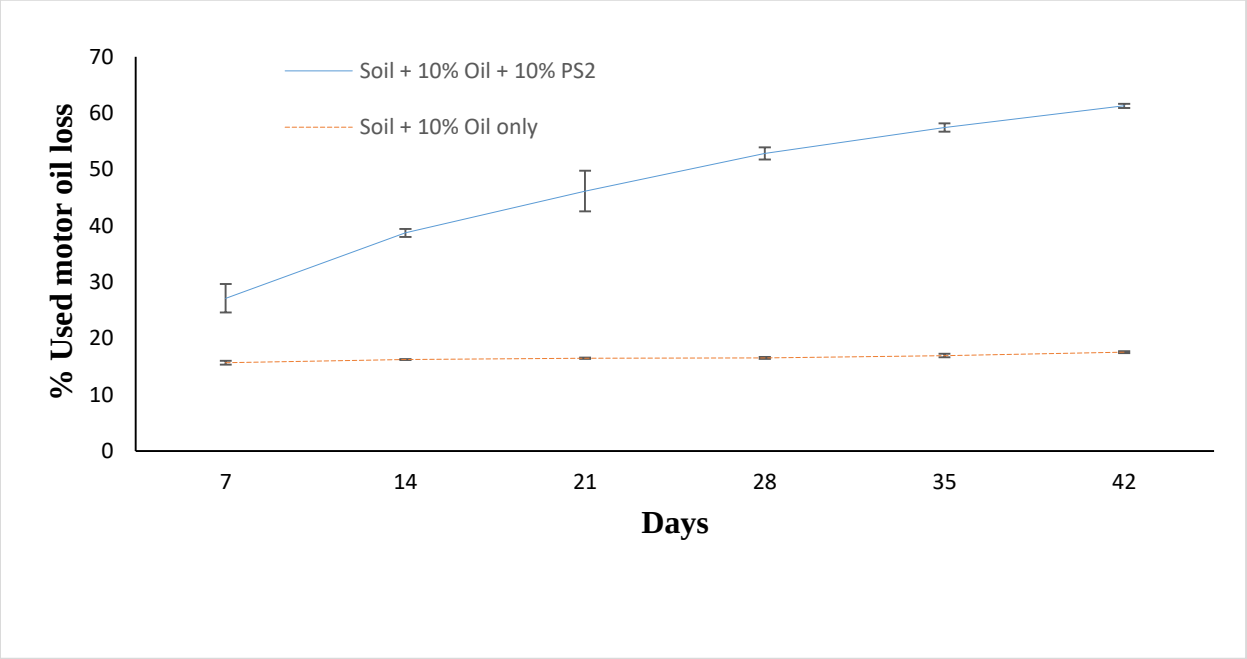

Fig.2: Bioremediation in soil polluted with 10%(v/w) used motor oil and amended with 10% (w/w) PS. Error bars indicate standard errors (n = 3).

The level of oil loss in the control and polluted soil amended with 15% PS over a 42-day period is presented in **Figure 3.** Percentage oil loss in the amended soil ranged from 40.52% to 73.85%.

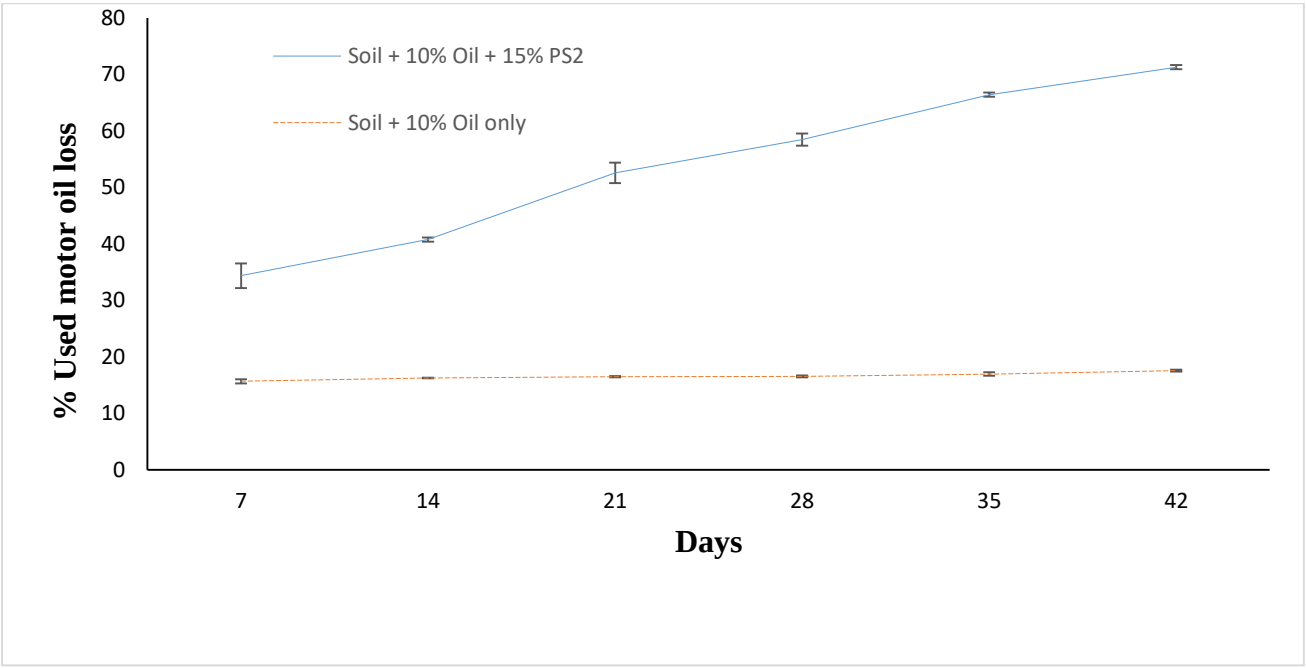

Fig.2: Bioremediation in soil polluted with 10% (v/w) used motor oil and amended with 10% (w/w) PS. Error bars indicate standard errors (n = 3).

Microbial Populations Recorded Throughout the Forty-Two-Day Period

Tables 2 shows the microbial populations in polluted control oil and polluted soil with three levels of amendment at day 0. Active aerobic heterotrophic bacterial colonies (AHB) were recorded in potato skin (PS)-amended soil, ranging from 9×10^7 to 17.2×10^7 CFU g^{-1} across all amendment levels. Unamended soil (control) gave AHB count 0.6×10^7CFU g^{-1} of soil. Similarly, hydrocarbon utilizing bacteria colonies (HUB) were recorded in PS-amended soil, ranging from 6.7×10^6 to 11.5×10^6CFU g^{-1} across all amendment levels. Unamended soil (control) gave HUB count of 0.2×10^6 CFU g^{-1} of soil. Furthermore, fungal population were recorded in PS-amended soil, ranging from 4.8×10^5 to 8.9×10^5 CFU g^{-1} across all amendment levels. Fungal count of $0.1 \times 10^5$was recorded in the unamended control soil.

Table 2: Microbial population on Day 0

Soil preparations	Colony forming units/gram		
	AHB	HUB	Fungi
Soil + 10% used motor oil + 5% potato skin	9.0×10^7	6.7×10^6	4.8×10^5
Soil + 10% used motor oil + 10% potato skin	11.0×10^7	8.3×10^6	5.3×10^5
Soil + 10% used motor oil + 15% potato skin	17.2×10^7	11.5×10^6	8.9×10^5
Soil + 10% used motor oil only	0.6×10^7	0.2×10^6	0.1×10^5

Microbial counts recorded on day 7 for the control soil and polluted soil with three levels of amendment are presented in **Table 3**. Active aerobic heterotrophic bacterial colonies (AHB) were recorded in potato skin (PS)-amended polluted soil, ranging from 10.8-23.7 $\times 10^7$ CFU g^{-1} across all amendment levels. Unamended soil (control) gave AHB count 1.85×10^7 CFU g^{-1} of soil. Similarly, hydrocarbon utilizing bacteria colonies (HUB) from the PS-amended option ranged from 8.1-13.8 $\times 10^6$ CFU g^{-1} across all amendment levels. Unamended soil (control) gave HUB count of 1.4×10^6 CFU g^{-1} of soil. Furthermore, fungal population recorded in PS-amended soil ranged from 4.9×10^5 to 10.1×10^5 CFU g^{-1} across all amendment levels. Fungal count of 0.6×10^5 was recorded in the unamended control soil

Table 3: Microbial population on Day 7

Soil preparations	Colony forming units/gram		
	AHB	HUB	Fungi
Soil + 10% used motor oil + 5% potato skin	10.8×10^7	8.1×10^6	4.9×10^5
Soil + 10% used motor oil + 10% potato skin	15.2×10^7	9.3×10^6	7.5×10^5
Soil + 10% used motor oil + 15% potato skin	23.7×10^7	13.8×10^6	10.1×10^5
Soil + 10% used motor oil only	1.85×10^7	1.4×10^6	0.6×10^5

Tables 4 shows the microbial populations in polluted control soil and polluted soil with three levels of amendment at days 14. Active aerobic heterotrophic bacterial colonies (AHB) were recorded in potato skin (PS)-amended polluted soil, ranging from 14.3×10^7 to 28.9×10^7 CFU g^{-1} across all amendment levels. Unamended soil (control) gave AHB count 3.7×10^7CFU g^{-1} of soil. Similarly, hydrocarbon utilizing bacteria colonies (HUB) were recorded in PS-amended soil, ranging from 9.8×10^6 to 15.3×10^6 CFU g^{-1}across all amendment levels. Unamended soil (control) gave HUB count of 2.9×10^6 CFU g^{-1} of soil. Furthermore, fungal population were recorded in PS-amended soil, ranging from 7.3×10^5 to 13.5×10^5 CFU g^{-1} across all amendment levels. Fungal count of 1.5×10^5 was recorded in the unamended control soil

Table 4: Microbial population on Day 14

Soil preparations	Colony forming units/gram		
	AHB	HUB	Fungi
Soil + 10% used motor oil + 5% potato skin	14.3×10^7	9.8×10^6	7.3×10^5
Soil + 10% used motor oil + 10% potato skin	18.4×10^7	10.8×10^6	9.3×10^5
Soil + 10% used motor oil + 15% potato skin	28.9×10^7	15.3×10^6	13.5×10^5
Soil + 10% used motor oil only	3.7×10^7	2.9×10^6	1.5×10^5

Microbial counts recorded on day 21 for the control soil and polluted soil with three levels of amendment are presented in **Table 5**. Active aerobic heterotrophic bacterial colonies (AHB) were recorded in potato skin (PS)-amended polluted soil, ranging from 25.7-67.0 × 10^7 CFU g^{-1} across all amendment levels. Unamended soil (control) gave AHB count 5.9 ×10^7CFU g^{-1} of soil. Similarly, hydrocarbon utilizing bacteria colonies (HUB) from the PS-amended option ranged from 11.9-17.1 × 10^6CFU g^{-1} across all amendment levels. Unamended soil (control) gave HUB count of 3.7×10^6 CFU g^{-1} of soil. Furthermore, fungal population recorded in PS-amended soil ranged from 13.9 × 10^5 to 23.1 × 10^5 CFU g^{-1} across all amendment levels. Fungal count of 2.1 × 10^5 was recorded in the unamended control soil.

Table 5: Microbial population on day 21

Soil preparations	Colony forming units/gram		
	AHB	HUB	Fungi
Soil + 10% used motor oil + 5% potato skin	25.7×10^7	11.9×10^6	13.9×10^5
Soil + 10% used motor oil + 10% potato skin	45.0×10^7	12.1×10^6	11.5×10^5
Soil + 10% used motor oil + 15% potato skin	67.0×10^7	17.1×10^6	23.1×10^5
Soil + 10% used motor oil only	5.9×10^7	3.7×10^6	2.1×10^5

Microbial populations recorded on day 21 for the control soil and polluted soil with three levels of amendment are presented in **Table 6**. Active aerobic heterotrophic bacterial colonies (AHB) were recorded in potato skin (PS)-amended polluted soil, ranging from 25.7-67.0 × 10^7 CFU g^{-1} across all amendment levels. Unamended soil (control) gave AHB count 5.9 ×10^7CFU g^{-1} of soil. Similarly, hydrocarbon utilizing bacteria colonies (HUB) from the PS-amended option ranged from 11.9-17.1 × 10^6CFU g^{-1} across all amendment levels. Unamended soil (control) gave HUB count of 3.7×10^6 CFU g^{-1} of soil. Furthermore, fungal population recorded in PS-amended soil ranged from 13.9 × 10^5 to 23.1 × 10^5 CFU g^{-1} across all amendment levels. Fungal count of 2.1 × 10^5 was recorded in the unamended control soil.

Table 6: Microbial population on day 28

Soil preparations	Colony forming units/gram		
	AHB	HUB	Fungi
Soil + 10% used motor oil + 5% potato skin	28.3×10^7	13.1×10^6	22.6×10^5
Soil + 10% used motor oil + 10% potato skin	55.0×10^7	14.7×10^6	26.3×10^5
Soil + 10% used motor oil + 15% potato skin	89.0×10^7	19.1×10^6	27.4×10^5
Soil + 10% used motor oil only	7.2×10^7	4.1×10^6	4.4×10^5

Tables 7 shows the microbial populations recorded in all the amended microcosms and the unamended control option on day 35. Active aerobic heterotrophic bacterial (AHB) counts recorded in potato skin (PS)-amended polluted soil ranged from 57.0×10^7 to 98.0×10^7 CFU g^{-1} across all amendment levels. AHB counts in the control option were 9.9×10^7CFU g^{-1} of soil. Similarly, HUB counts recorded in PS-amended soil ranged from 16.7×10^6 to 21.1×10^6 CFU g^{-1} across all amendment levels. Unamended soil (control) gave HUB count of 6.3×10^6 CFU g^{-1} of soil. Furthermore, fungal population were recorded in PS-amended soil, ranging from 38.0×10^5 to 51.0×10^5 CFU g^{-1} across all amendment levels. Fungal count of 5.7×10^5 was recorded in the unamended control soil.

Table 7: Microbial population on Day 35

Soil preparations	Colony forming units/gram		
	AHB	HUB	Fungi
Soil + 10% used motor oil + 5% potato skin	57.0×10^7	16.7×10^6	38.0×10^5
Soil + 10% used motor oil + 10% potato skin	63.0×10^7	17.1×10^6	45.0×10^5
Soil + 10% used motor oil + 15% potato skin	98.0×10^7	21.1×10^6	51.0×10^5
Soil + 10% used motor oil only	9.9×10^7	6.3×10^6	5.7×10^5

Microbial counts recorded on day 42 for the control soil and polluted soil with three levels of amendment are presented in **Table 8**. Active aerobic heterotrophic bacterial colonies (AHB) were recorded in potato skin (PS)-amended polluted soil, ranging from 53.0-106.0 $\times 10^7$ CFU g^{-1} across all amendment levels. Unamended soil (control) gave AHB counts of 10.7×10^7CFU g^{-1} of soil. Similarly, HUB counts from the PS-amended option ranged from 14.9-22.3 $\times 10^6$ CFU g^{-1}across all amendment levels. Unamended soil (control) gave HUB count of 7.1×10^6 CFU g^{-1} of soil. Furthermore, fungal population recorded in PS-amended soil ranged from 39.0×10^5 to 59.0×10^5 CFU g^{-1} across all amendment levels. Fungal count of 6.4×10^5 was recorded in the unamended control soil.

Table 8: Microbial population on Day 42

Soil preparations	Colony forming units/gram		
	AHB	HUB	Fungi
Soil + 10% used motor oil + 5% potato skin	53.0×10^7	14.9×10^6	39.0×10^5
Soil + 10% used motor oil + 10% potato skin	71.0×10^7	19.9×10^6	51.0×10^5
Soil + 10% used motor oil + 15% potato skin	106.0×10^7	22.3×10^6	59.0×10^5
Soil + 10% used motor oil only	10.7×10^7	7.1×10^6	6.4×10^5

Isolates Identified

The identity of hydrocarbon-utilizing bacteria isolated from both the control soil and amended soil throughout the 42-day period are presented in **Tables 9 and 10**. Six hydrocarbon-utilizing bacteria belonging to the genera *Bacillus*, *Arthrobacter*, *Rhodococcus*, *Corynebacterium*, *Pseudomonas* and *Acinetobacter* were predominantly isolated based on their Gram reaction and biochemical characteristics. Similarly, **Figure 4** presents the identity of the fungal isolates. The fungi include: *Aspergillus niger*, *Aspergillus* sp., *Pennicillium* sp., *Phialophora* sp., *Cladosporium* sp. and *Verticillum* sp.

Table 9. Morphological and microscopic characteristics of bacterial isolates.

Isolates	Morphological characteristics	Microscopic characteristics
SWO_a	Large, Round, Irregular, Flat, Milky, Smooth,	Small, Gram Positive, Rods
SWO_b	Small, Round, Irregular, Flat, Greenish, Smooth, Translucent	Small, Gram Positive, Staph
SPS_a	Large, Round, Irregular, Flat, Milky, Smooth,	Small, Gram Positive, Rods
SPS_b	Pin Point, Round, Entire, Flat, Greenish, Smooth, Opaque	Small, Gram Positive, Cocci
SPS_c	Small, Round, Irregular, Flat, Greenish, Smooth, Translucent	Small, Gram Positive, Staph
SPS_d	Large, Opaque, Irregular Creamy	Small, Gram Positive, Rods

SWO: soil with used motor oil, **SPS**: polluted soil amended with potato skin

Table 10. Biochemical characteristics of bacteria Isolates

Isolates	SWOa	SWOb	SPSa	SPSb	SPSc	SPSd
Gram reaction	+	+	+	+	-	+
Starch hydrolysis	+	-	+	-	-	+
Citrate utilisation	-	+	-	+	+	+
Urease production	-	-	-	+	-	+
indole	-	-	-	-	-	-
H_2S	-	-	-	+	-	-

Methyl-red	+	-	+	+	-	-
Voges-Proskauer	-	-	-	-	-	-
Oxidase	-	-	-	+	-	-
catalase	+	+	+	+	+	+
motility	+	-	+	-	-	-
Spore formation	+	-	+	-	-	-
Probable identity	*Bacillus* sp	*Staphylococcus* sp	*Bacillus* sp	*Micrococcus* sp	*Staphylococcus* sp	*Arthrobacter* sp

Key: Negative (-); Positive (+); **SWO**: soil with used motor oil, **SPS**: soil amended with potato ski

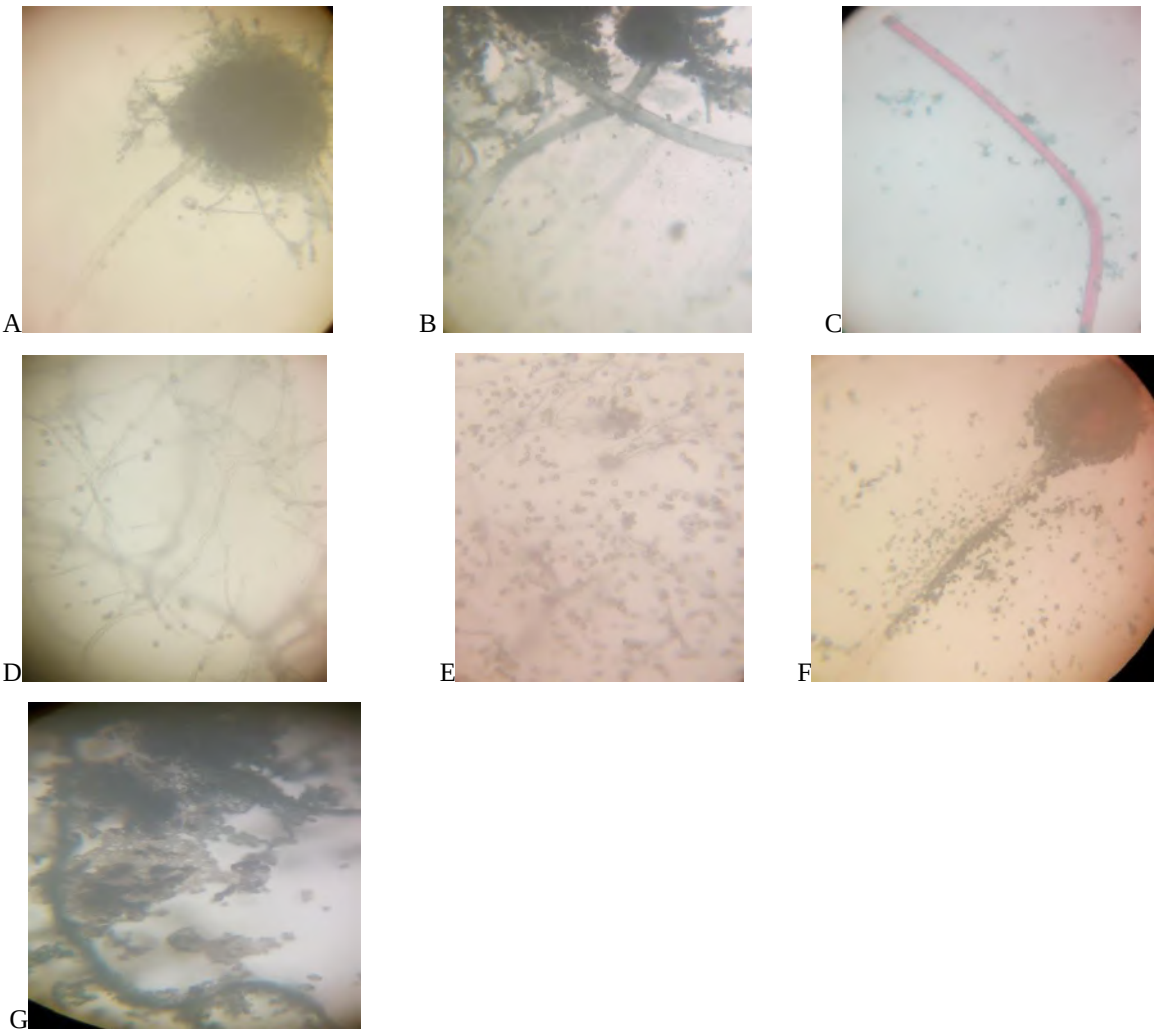

Fig.4: Fungal reproductive structures in Sabouraud dextrose agar. A: Filamentous fungus – Aspergillus niger; B: Conidiophores with spore masses of Cladosporium sp.; C: Pink conidiophore and spores of Fusarium sp.; D: Conidiophore, phialides and conidia of Phialophora sp.; E Penicillium sp.; F: Aspergillus fumigatus; G: Thick walled resting cells of Verticillum sp.

Seed Germination Toxicity and Germination Index

The results of germination toxicity test with *Cucumis sativum* for positive control soil, negative control soil and polluted soil across all levels of amendment are presented in **Table 11**. Percentage SG ranged from 50 to 75 across all amendment levels, %GR ranged from 65.65-92.16 across all amendment levels while GI values ranged from 38.83 to 69.46 across all amendment levels. Positive and negative controls had GI of 100 and 3.80 respectively.

Table 11: Seed Germination Parameters of Control and Amended Soils

Soil preparations	SG	GR(cm)	%SG	%GR	GI
Soil + 10% used motor oil + 5% potato skin	4.00	1.51	50.00	65.65	38.83
Soil + 10% used motor oil + 10% potato skin	5.00	1.67	62.50	72.61	45.38
Soil + 10% used motor oil + 15% potato skin	6.00	2.13	75.00	92.16	69.46
Soil + 10% used motor oil (negative control)	1.00	0.70	12.5	30.43	3.80
Soil with no oil contamination (positive control)	8.00	2.30	100.00	100.00	100.00

SG: Number of seeds that germinated; GR: Root length; GI: Germination index

IV. DISCUSSION

A review of studies on bioremediation of hydrocarbon contaminated sites reveals a growing concern by scientists to understand better ways of treating soils contaminated with petroleum products. In the present study, the remediation of used motor oil-contaminated soil by the use potato skin amendment to stimulate microbial activity was explored. A good number of microorganisms, including bacteria and fungi, could be found in the soil. In UMO-polluted soil, bacteria and fungi are continuously exposed to UMO, giving rise to survival of bacteria and fungi that can utilize the same. The data presented in this research were limited to laboratory experiments. The results from our study showed significant reduction in the level of used motor oil contamination in the potato skin-amended soil

In the present study, potato skin had higher nitrogen content than the experimental soil (**Table 1**). Nitrogen and phosphorus have been reported as essential nutrients for bioremediation of petroleum hydrocarbons in the soil [19]. In other words, as bioremediation progresses in the absence of external nutrient sources such as nitrogen, carbon, phosphorus etc., the microorganisms will utilize available nutrients in the soil to a point of depletion and the nutrients becomes limiting. The soil pH (7.03±1.15) **(Table 1)** was within the acceptable limits required for effective biodegradation [20].

The result of the extraction efficiency experiment clearly revealed that diethylether was the best choice in the extraction of crude oil among the other two solvents, namely dichloromethane and n-hexane. This is due to the highest percentage extraction (89%) observed with diethylether.

In the present study, reduction in Total Petroleum Hydrocarbons (TPH) increased appreciably as the weeks of biodegradation increased. Oil loss increased notably (between 27.18% and 73.85%) throughout the 42-day period across all amendment levels **(Figure 1-3)**. Highest oil loss was observed in the highest amendment level (15%) **(Figure 3)**. The observed enhancement in oil loss at 15% amendment level was probably due to the enhanced level of nutrients at that level of amendment. It was reported that TPH removal always increases as the days of incubation increases [21]. In a similar vein, Abdulsalam [22] also reported an increase in removal of TPH in soil contaminated with used motor oil as the incubation period was elongated.

Oil loss in the control option was also progressive throughout the study period but was much slower **(Figure 1-3).** Mechanisms such as photodegradation, volatilization, sorption and bioattenuation by the indigenous hydrocarbonoclastic flora might have played some contributory roles to the observed trend in the control microcosm. Similar works recorded low TPH loss in the control option [23, 24]

Critical evaluation and assessment of reports on biostimulation-based bioremediation processes reveal some level of inconsistency. While some have demonstrated direct relationship between TPH loss and organic amendment [25, 26], a scenario where natural attenuation in the unamended soil was more successful than biostimulation has been

reported [27]. Also, there was no significant effect of nutrient amendment in the work carried out by Seklemova [28]. It stands to reason therefore, that soils have varying degrees of microbial potentials as touching degradation of hydrocarbon pollutants.

Microbial growth and activities can be used as a probe for impact of organic wastes [23]. In the present work, the microbial counts study revealed that AHB, HUB and fungal populations increased appreciably in each successive week, AHB populations being the greatest in each week. It was also observed that HUB populations were greater than their fungal counterparts all throughout the study period (**Table 6**). Among the bacteria groups, HUB was found to be lower than the AHB. It might be deduced from the observed trend that HUB are a group of AHB that evolved due to incessant hydrocarbon spills. In a similar manner, it was noted that fungal groups were lower in number than their hydrocarbon-degrading bacterial counterparts (**Tables 2-8**). Chikere *et al* [29] argued that even while it has been generally accepted that bacteria fungi and bacteria are the major microbial groups involved in hydrocarbon remediation, bacteria have been found to be more versatile and therefore participate more frequently in several microbial remediation processes. Higher TPH loss and microbial levels were observed in the amended options. Similar trend has been widely documented [30, 31, 32]. In another sense, the observation of higher microbial counts and TPH loss in the amended options might be due to the varying groups of microbes associated with potato skin with innate hydrocarbon-degrading abilities.

Tentative identification of the hydrocarbon-utilizing bacteria isolated in the present study revealed the presence of bacteria belonging to the genera: *Bacillus, Arthrobacter, Rhodococcus, Corynebacterium, Pseudomonas, Staphylococcus* and *Acinetobacter*. Earlier researchers have documented these bacteria as having potential hydrocarbon-remediation attributes [33,34]. Among the bacteria, *Bacillus* was predominantly isolated in this work **(Tables9-10)**. The ability of *Bacillus* isolated from Nigerian soil has consistently been observed and attributed to competent hydrocarbon degrading enzyme system of the organism, its ability to form spores and emulsify crude oil [26]. Similarly, the fungi isolated were tentatively identified as *Aspergillus niger*, *Aspergillus* sp., *Pennicillum* sp., *Phialophora* sp., *Cladosporium* sp. and *Verticillum* sp. **(Figure 4)**. Adekunle and Adeniyi [35] reported species of *Penicillum* and *Aspergillus* that degrade kerosene, spent engine oil, unspent engine oil, diesel and extracted oil from *Treculia africana* seed.

Cucumber seed is sensitive to toxic chemicals (mostly petroleum hydrocarbons) and it is an important agricultural crop, which led to its wide use for toxicity test and as a bioindicator [17]. Millioli *et al.* [36] reported a decrease in the number of germinated seeds with 10% petroleum contamination in the soil. Germination index (GI) of soil treated with 15% PS gave the highest value of 69.46 (**Table 11)**. However, the GI of plants grown on untreated contaminated soil was very low, signifying low bioremediation in the treatment option. Hydrocarbons may affect root surface, preventing or reducing gas and water exchange and nutrient absorption. Hydrocarbons may also enter the seeds and alter the metabolic reactions and kill the embryo. Hydrocarbons damage cell membranes and reduce the metabolic transport and respiration rate [37, 38]. In the present study, variations in plant growth parametres in the different soil preparations have shown that soil quality can affect productivity. In a 126-day study using soy cake, potato skin, and tea leaf amendments, Agamuthu and Dadrasnia [25] reported higher percentage seed germination of 90%, 70%, and 60%, respectively with seeds of lettuce (*Lactuca sativa L.*) in just seven days after treatment. Ogboghodo [37] and Oleszczuk [38] reported that low percentage germination and low germination index is sequel to low biodegradation of oil or short treatment of oil-contaminated soil. From the present study, it took the cucumber seeds a minimum of 21 days to germinate instead of the normal range of 7-10 days; this signifies that cucumber seeds are very sensitive to used motor oil. Growth of all seeds planted was recorded in the positive control while a lower percentage germination recorded in the negative control signified that germination of seeds can go undisturbed in a hydrocarbon-free soil. There was a significant difference in bioremediation level between the control and amended soil even at 5% amendment level.

V. CONCLUSION

Used motor oil pollution of soil proved to negatively alter the soil quality, depressing aerobic heterotrophic bacterial counts and encouraging the proliferation of oil utilizing bacteria in the soil. Amendment of UMO-polluted soil with potato skin caused proliferation of oil-degrading microbes and enhanced microbial degradation of used motor oil in the soil. Potato skin might have provided alternative source of N and P, to stimulate microbial activity. The study

therefore shows the viability of using potato skin amendment in remediating hydrocarbon-contaminated soil. Potato skin therefore affords an alternative method in removing used motor oil contaminants from soil.

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